CLAIMS

What is claimed is:

1. A method for the determination of the functional effect of a test agent on a nuclear receptor protein or an active fragment thereof comprising the steps of: (A) combining in a first *in vitro* reaction cocktail said nuclear receptor protein or said active fragment thereof fused to a purification facilitating compound; a nuclear receptor coregulator protein or an active fragment thereof fused to an enzyme or a fragment thereof whose activity is simply quantified; a ligand for said nuclear receptor protein; a purification facilitating partner affixed to a solid support; and said test agent; (B) incubating the components of step (A) to allow said components to form a complex; (C) removing said solid support from the remainder of said first *in vitro* reaction cocktail; (D) determining an amount of said complex that was formed by assaying said removed solid support for a first activity of said enzyme or fragment thereof; and (E) assessing whether said test agent functioned as an effector of complex formation by comparing said first activity to a second activity from said enzyme or fragment thereof, recovered from a second *in vitro* reaction cocktail comprising all of the components of step (A) except said test agent, where said second *in vitro* reaction cocktail was subjected to steps (B) to (D).

2. The method as defined in claim **1** wherein said test agent is a protein, peptide, nucleic acid, hormone, cytokine, lipid, carbohydrate, vitamin, mineral, large organic molecule, small organic molecule, non-organic agent or any combination thereof.

3. The method as defined in claim **1** wherein said nuclear receptor is a steroid receptor.

4. The method as defined in claim **1** wherein said nuclear receptor is a non-steroid receptor.

5. The method as defined in claim **1** wherein said nuclear receptor is a peroxisome proliferator-activated receptor, thyroid receptor, estrogen receptor, glucocorticoid receptor, progesterone receptor, androgen receptor, mineralcorticoid

receptor, retinoic acid receptor, retinoid X receptor, vitamin D receptor, orphan receptor, any fragment thereof or any combination thereof.

6. The method as defined in claim **1** wherein said active fragment of said nuclear receptor comprises the ligand binding domain.

7. The method as defined in claim **5** wherein said active fragment of said nuclear receptor comprises the ligand binding domain.

8. The method as defined in claim **1** wherein said active fragment of said nuclear receptor coregulator comprises one or more LXXLL motifs.

9. The method as defined in claim **1** wherein said purification facilitating compound is glutathione-S-transferase, maltose K, influenza hemaglutinin, avidin, biotin, FLAG, myc tag, histidine multimers, or any combination thereof.

10. The method as defined in claim **1** wherein said purification facilitating partner is glutathione, maltose, anti-influenza hemaglutinin antibodies, avidin, biotin, anti-FLAG antibodies, anti-myc antibodies, ionic nickel, or any combination thereof.

11. The method as defined in claim **1** wherein said nuclear receptor coregulator is a nuclear receptor coactivator.

12. The method as defined in claim **11** wherein said nuclear receptor coactivator is a steroid receptor coactivator-1, steroid receptor coactivator-2, steroid receptor coactivator-3, transcription intermediary factor 2, glucocorticoid receptor interacting protein 1, retinoic acid receptor interacting protein 3, coactivator-associated arginine methyltransferase 1, peroxisome proliferator-activated receptor gamma coactivator-1, peroxisome proliferator-activated receptor gamma coactivator-2, p300/CREB binding protein, p300, CREB-binding protein-interacting protein, nuclear-receptor co-activator protein, p300/CBP-associated factor, alteration/deficiency in activation 3 protein, small nuclear RING finger protein, thyroid hormone receptor-associated protein 220, NR-binding SET-domain-containing protein, any fragment thereof, or any combination thereof.

13. The method as defined in claim **1** wherein said nuclear receptor coregulator is a nuclear receptor corepressor.

14. The method as defined in claim **13** wherein said nuclear receptor corepressor is nuclear receptor corepressor (N-Cor), small ubiquitous nuclear corepressor, silencing mediator for retinoic acid and thyroid hormone receptors,

transcription intermediary factor 2, thyroid hormone receptor uncoupling protein, calreticulin, repressor of estrogen receptor activity, NR-binding SET-domain-containing protein, any fragment thereof, or any combination thereof.

15. The method as defined in claim **1** wherein said solid support is a glass bead, cellulose bead, polystyrene bead, sephadex bead, sepharose bead, polyacrylamide bead, agarose bead, magnetic bead, multi-well plate, glass reaction vessel, or plastic reaction vessel.

16. The method as defined in claim **1** wherein said enzyme is luciferase, β-galactosidase, alkaline phosphatase, peroxidase, chloramphenicol acetyl transferase or green fluorescent protein.

17. The method as defined in claim **1** wherein said second *in vitro* reaction cocktail comprises a control agent known to have an effect on said nuclear receptor.

18. The method as defined in claim **5** wherein said second *in vitro* reaction cocktail comprises a control agent known to have an effect on said nuclear receptor.

19. The method as defined in claim **1** wherein said first *in vitro* reaction cocktail comprises multiple nuclear receptors or active fragments thereof essentially simultaneously and said method comprises an additional step (f) comprising deconvoluting the active nuclear receptor after assessing whether said test agent functioned as an effector of the nuclear receptor protein.

20. The method as defined in claim **1** wherein said test agent functioned as an agonist of complex formation, an antagonist of complex formation, or a ligand for said nuclear receptor protein.

21. A high-throughput assay comprising the method as defined in claim **1** and utilizing multiple *in vitro* reaction cocktails for the determination of the functional effect of multiple test agents on a nuclear receptor protein or a fragment thereof.

22. A method for the identification of a nuclear receptor ligand, comprising the steps of: (A) combining in a first *in vitro* reaction cocktail a nuclear receptor protein or an active fragment thereof fused to a purification facilitating compound; a nuclear receptor coregulator protein or an active fragment thereof fused to an enzyme or a fragment thereof whose activity is simply quantified; a

purification facilitating partner affixed to a solid support; and a test agent; (B) incubating the components of step (A) to allow the components to form a complex; (C) removing said solid support from the remainder of said first *in vitro* reaction cocktail; (D) determining an amount of complex that was formed by assaying said removed solid support for a first activity of the enzyme or fragment thereof; and (E) assessing whether said test agent functioned as a ligand for the nuclear receptor or active fragment thereof by comparing said first activity to a second activity from said enzyme or fragment thereof recovered from a second *in vitro* reaction cocktail comprising all of the components of step (A) except said test agent, where said second *in vitro* reaction cocktail was subjected to steps (B) to (D).

23. The method as defined in claim **22** wherein said test agent is a protein, peptide, nucleic acid, hormone, cytokine, lipid, carbohydrate, vitamin, mineral, large organic molecule, small organic molecule, non-organic agent or any combination thereof.

24. The method as defined in claim **22** wherein said nuclear receptor is a steroid receptor.

25. The method as defined in claim **22** wherein said nuclear receptor is a non-steroid receptor.

26. The method as defined in claim **22** wherein said nuclear receptor is peroxisome proliferator-activated receptor, thyroid receptor, estrogen receptor, glucocorticoid receptor, progesterone receptor, androgen receptor, mineralcorticoid receptor, retinoic acid receptor, retinoid X receptor, vitamin D receptor, orphan receptor, any fragment thereof, or any combination thereof.

27. The method as defined in claim **22** wherein said active fragment of said nuclear receptor comprises the ligand binding domain.

28. The method as defined in claim **26** wherein said active fragment of said nuclear receptor comprises the ligand binding domain.

29. The method as defined in claim **22** wherein said active fragment of said nuclear receptor coregulator comprises one or more LXXLL motifs.

30. The method as defined in claim **22** wherein said purification facilitating compound is glutathione-S-transferase, maltose K, influenza hemaglutinin, avidin, biotin, FLAG, myc tag or histidine multimers.

31. The method as defined in claim **22** wherein said purification facilitating partner is glutathione, maltose, anti-influenza hemaglutinin antibodies, avidin, biotin, anti-FLAG antibodies, anti-myc antibodies, ionic nickel, or any combination thereof.

32. The method as defined in claim **22** wherein said nuclear receptor coregulator is a nuclear receptor coactivator.

33. The method as defined in claim **32** wherein said nuclear receptor coactivator is steroid receptor coactivator-1, steroid receptor coactivator-2, steroid receptor coactivator-3, transcription intermediary factor 2, glucocorticoid receptor interacting protein 1, retinoic acid receptor interacting protein 3, coactivator-associated arginine methyltransferase 1, peroxisome proliferator-activated receptor gamma coactivator-1, peroxisome proliferator-activated receptor gamma coactivator-2, p300/CREB binding protein, p300, CREB-binding protein-interacting protein, nuclear-receptor co-activator protein, p300/CBP-associated factor, alteration/deficiency in activation 3 protein, small nuclear RING finger protein, thyroid hormone receptor-associated protein 220, NR-binding SET-domain-containing protein, any fragment thereof, or any combination thereof.

34. The method as defined in claim **22** wherein said nuclear receptor coregulator is a nuclear receptor corepressor.

35. The method as defined in claim **34** wherein said nuclear receptor corepressor is nuclear receptor corepressor (N-Cor), small ubiquitous nuclear corepressor, silencing mediator for retinoic acid and thyroid hormone receptors, transcription intermediary factor 2, thyroid hormone receptor uncoupling protein, calreticulin, repressor of estrogen receptor activity, NR-binding SET-domain-containing protein, any fragment thereof or any combination thereof.

36. The method of claim **22** wherein said solid support is a glass bead, cellulose bead, polystyrene bead, sephadex bead, sepharose bead, polyacrylamide bead, agarose bead, magnetic bead, multi-well plate, glass reaction vessel or plastic reaction vessels.

37. The method of claim **22** wherein said enzyme is luciferase, β-galactosidase, alkaline phosphatase, peroxidase, chloramphenicol acetyl transferase, and green fluorescent protein.

38. The method as defined in claim **22** wherein said second *in vitro* reaction cocktail comprises a control agent known to be a ligand for said nuclear receptor or active fragment thereof.

39. A high-throughput assay comprising the method as defined in claim **22** and utilizing multiple *in vitro* reaction cocktails for the identification of a ligand for a nuclear receptor protein or a fragment thereof.

40. A method for the determination of a functional effect of a test agent on a peroxisome proliferator-activated receptor protein or a fragment thereof, comprising the steps of: (A) combining in a first *in vitro* reaction cocktail said peroxisome proliferator-activated receptor protein or said fragment thereof fused to the glutathione-S-transferase protein or a fragment thereof; a steroid receptor coactivator-1 protein or a fragment thereof fused to the luciferase protein or a fragment thereof; a GW2331 ligand; glutathione-sepharose beads; and said test agent; (B) incubating the components of step (A) to allow said components to form a complex; (C) removing said glutathione-sepharose beads from the remainder of said first *in vitro* reaction cocktail; (D) determining an amount of said complex that was formed by assaying said removed glutathione-sepharose beads for a first luciferase activity; and (E) assessing whether said test agent functioned as an effector of complex formation by comparing said first luciferase activity to a second luciferase activity recovered from a second *in vitro* reaction cocktail comprising all of the components of step (A) except said test agent, where said second *in vitro* reaction cocktail was subjected to steps (B) to (D).

41. A method for the identification of a ligand for a peroxisome proliferator-activated receptor protein or a fragment thereof, comprising the steps of: (A) combining in a first *in vitro* reaction cocktail said peroxisome proliferator-activated receptor protein or said fragment thereof fused to the glutathione-S-transferase protein or a fragment thereof; a steroid receptor coactivator-1 protein or a fragment thereof fused to the luciferase protein or a fragment thereof; glutathione-sepharose beads; and a test agent; (B) incubating the components of step (A) to allow said components to form a complex; (C) removing said glutathione-sepharose beads from the remainder of said first *in vitro* reaction cocktail; (D) determining an amount of said complex that was formed by assaying

said removed glutathione-sepharose beads for a first luciferase activity; and (E) assessing whether said test agent functioned as a ligand for peroxisome proliferator-activated receptor protein by comparing said first luciferase activity to a second luciferase activity recovered from a second *in vitro* reaction cocktail comprising all of the components of step (A) except said test agent, where said second in vitro cocktail was subjected to steps (B) to (D).

42. A pharmaceutical composition comprising an effector as defined in claim **1** and a pharmaceutically acceptable carrier, vehicle, or diluent.